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Spectroscopic study and G-quadruplex DNA binding affinity of two bioactive papaverine-derived ligands

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Abstract

The interactions of G-quadruplex DNA with two oxidation products of papaverine, 6a,12a-diazadibenzo-[*a*,*g*]fluorenylium derivative (**1**) and 2,3,9,10-tetramethoxy-12-oxo-12H-indolo[2,1-*a*]isoquinolinium cation (**2**) were investigated. Their activity against telomerase was assessed using the conventional telomeric repeat amplification protocol (TRAP) assay. Effect of TRAP buffer and oligonucleotide length on the DNA-binding affinity of **1** and **2** were also studied. Three quadruplex-forming oligonucleotides with human telomeric sequence: $dG_3(T_2AG_3)_3$ (htel21), $dAG_3(T_2AG_3)_3$ (htel22), and $d(T_2AG_3)_4$ (htel24) were used in these investigations. Both ligands were capable of interacting with G4 DNA with binding stoichiometry indicating that two ligand molecules bind to G-quadruplex, which agrees with the binding model of end-stacking on terminal G-tetrads. Circular dichroism spectra revealed that preferences of quadruplex-forming oligonucleotide to adopt a particular topological structure may be also affected by the external ligand that binds to quadruplex. Telomerase activity was suppressed at very low ligand **1** and ligand **2** concentrations with an appreciable selectivity comparing with inhibition of Taq polymerase.

Keywords: DNA binding; G-quadruplex DNA; Papaverine derivatives; Telomerase inhibitor

1. Introduction

Telomeres are specialized DNA/protein structures found at the end of all eukaryotic chromosomes. Mammalian telomeric DNA comprises tandem repeats of double-stranded track of guanine-rich sequences, (TTAGGG/CCCTAA), and a short, single-stranded G-rich 3′-overhang [1]. The 3′-overhang may be involved in several DNA conformations such as T-loops [2], triplexes [3], or G-quadruplexes (G4) [4]. Folding of the G-rich 3′-overhang into G4 structure has been found to inhibit telomerase activity [5]. Telomerase, a reverse transcriptase, is able to maintain stable length of telomeric DNA that is essential in cellular immortalization [5,6]. Telomerase activity has been detected in some 80–90% of all human cancers but not in adjacent normal cells [7]. Consequently, telomerase activity is recognized as an important factor in tumorigenesis, thus has been proposed as a potentially highly selective target for the development of a novel class of anticancer agents [8,9].

A number of small molecules have been reported in recent years that stabilize G-quadruplex structures and inhibit telomerase activity [4,5,8–11]. Some of these molecules have been shown to induce telomere shortening and instability, triggering apoptosis in various tumor cell lines [12]. The mechanism of action of these ligands remains unclear since the in vivo existence of G-quadruplexes in human cells has not been yet definitely demonstrated.

In vitro characterization of G-quadruplexes indicates four-stranded structures containing one or more nucleic acid strands, in parallel or antiparallel orientations, stabilized by coordination of a monovalent cation within its central channel [13,14]. Four guanines on a plane interacting via Hoogsteen bonding form a G-quartet (Fig. 1a). Typically, three or four G-quartets are stacked and held together by π–π nonbonded attractive interactions, thus forming G-quadruplexes with different topological structures as shown in Fig. 1b–e.

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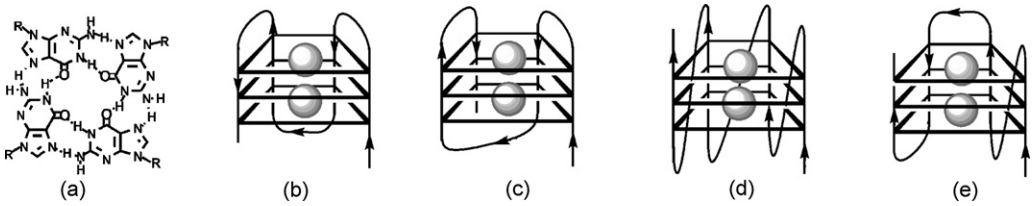

Fig. 1. Structures of G-tetrad (a) and the four types of intramolecular G-quadruplexes: antiparallel chair-type (b) and basket-type (c), parallel propeller-type (d), and hybrid mixed-type (e). Encapsulated metal cations are shown as balls.

Recently, we have reported on the new G-quadruplex binding ligands, two papaverine derivatives (ligand **1** and **2** in Fig. 2) [15–17]. These ligands showed significant binding affinity to G4 structures [16,17], aggregation tendency in aqueous solutions [16] and promising cytotoxicity with IC_{50} in micromolar concentration range [17]. In the current studies we use spectral techniques (CD and UV–vis spectroscopy) to study binding affinity of ligands to G-quadruplex DNA under the TRAP assay buffer conditions. The effect of the length of G4-forming oligonucleotide on ligand/G-quadruplex interactions has been also investigated. Furthermore, we report the ligand-induced inhibition of telomerase activity assessed by a telomerase repeat amplification protocol (TRAP) assay.

2. Materials and methods

The 6a,12a-diazadibenzo-[*a*,*g*]fluorenylium derivative and 2,3,9,10-tetramethoxy-12-oxo-12H-indolo[2,1-*a*]isoquinolinium chloride (ligand **1** and **2** in Fig. 2, respectively) were prepared according to the procedures described elsewhere [18,19]. The stock solutions (ca. 2.5 mM) were prepared in pure EtOH (ligand **1**) or in 50% (v/v) EtOH (ligand **2**); working solutions were prepared by dilution with pure water. The quadruplex-forming DNA oligonucleotides with human telomeric sequence: $dG_3(T_2AG_3)_3$ (htel21), $dAG_3(T_2AG_3)_3$ (htel22), and $d(T_2AG_3)_4$ (htel24) were synthesized and HPLC-purified by Genosys, Sigma or by Inst. Biochem. Biophys. PAN (Warsaw, Poland). All titration experiments were performed in a TRAP buffer (20 mM Tris buffer pH 8.3, 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.5% Tween 25 (polyoxyethylenesorbitan monolaurate)) containing 5% EtOH (v/v) unless otherwise noted. A Milli-Q filtered water (Millipore Co.) was used throughout.

Absorption spectra were recorded with a Specord M40 (Jena, Germany) or a Hewlett Packard model HP 845× spectrophotometer. All measurements were carried out using a 10 mm quartz cell. Typical titration consisted of successive replacement of small amounts (2–4 μl) of a 10 μM solution of dye in buffer by the same volume of concentrated oligonucleotide solution containing buffer and dye, followed by stirring, thermal equilibration, and recording of the spectrum. The data represented as binding isotherm, were fitted to the simple Scatchard equation [20].

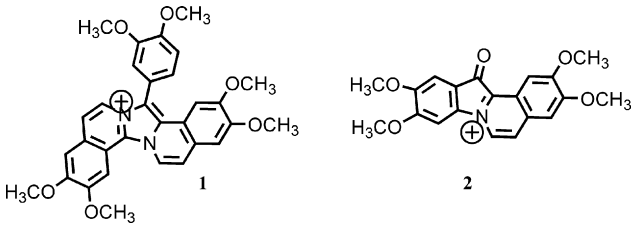

Fig. 2. Structures of the papaverine-derived ligands.

Circular dichroism (CD) spectra were recorded in the 200–500 nm spectral range with a Jasco J-810 spectropolarimeter at 25 °C using a quartz cell with a 10 mm path length. Sample solution in CD experiments contained 2.5 μM of oligonucleotide in TRAP buffer and the ligand was added up to a molar ratio r = drug/quadruplex = 10. Measurement of CD spectra were repeated at 5 μM of oligonucleotide in the range 330–550 nm for the systems exhibiting induced CD activity (ligand **1** with htel21, htel22 and htel24). CD Spectra result from the averaging of three scans, followed by subtraction of the CD spectrum of a buffer solution and smoothing. Results are expressed in molar ellipticity values.

The ability of ligands to inhibit telomerase in a cell-free assay was assessed using the quantitative TeloTAGGG Telomerase PCR ELISA^{PLUS} kit (Roche Molecular Biochemicals), which is an extension of the original telomeric repeat amplification protocol (TRAP) [21]. Initially, a protein extract was prepared from exponentially growing HL-60 cells (human promyelocytic leukemia) according to manufacturer's protocol for the TeloTAGGG Telomerase PCR ELISA^{PLUS} kit analysis. For each assay 2 μl of cell lysis extract (corresponding to 2000 cells) was used. Briefly, reaction mixtures contained telomerase extract, investigated ligand **1** or **2** in different concentrations, oligonucleotide primers (P1-TS and P2) for amplification of telomeric repeats and internal standard (IS), Taq DNA polymerase (5 units/μl) (Roche), dNTP Mix and TRAP reaction buffer. After 20 min incubation at 25 °C for telomerase extension of the P1-TS primer, the PCR cycling conditions were 94 °C for 5 min followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 90 s with final step at 72 °C for 10 min. The ELISA kit allows the highly specific amplification of telomerase-mediated elongation products combined with non-radioactive detection following an ELISA protocol. The levels of telomerase activity were within the linear range of the TRAP assay.

3. Results and discussion

3.1. Spectral properties of ligands

The effect of TRAP buffer on the spectral characteristics of ligands was examined in order to elucidate the tendency of lig-